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ARTICLE

Influences of Habitat and Hybridization on the Genetic Structure of Redband Trout in the Upper Snake River Basin, Idaho

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Abstract

The genetic structure of redband trout *Oncorhynchus mykiss gairdnerii* in the upper Snake River basin was investigated at various scales using 13 microsatellite loci. The majority of the genetic variation was partitioned between streams, although differentiation among watersheds was significant. This diversity was probably historically partitioned at the watershed scale when steelhead *O. mykiss* (anadromous rainbow trout) were present, with the exception of small, isolated, headwater streams where there may have been only resident trout. Genetic structure appears to have been altered by a combination of factors, including habitat fragmentation and hybridization with hatchery trout. Redband trout populations in the desert and montane environments both experienced reduced gene flow, but the desert populations displayed higher degrees of genetic differentiation. There was also a significant inverse relationship between the degree of genetic differentiation and the level of allelic diversity. Interspecific hybrids with cutthroat trout *O. clarkii* were detected within 9% of the sampled sites, but they made up only 2% of fish and were mostly confined to one sample location. In contrast, intraspecific hybrids with coastal rainbow trout *O. m. irideus* were detected within 31% of the samples sites and were more than twice as likely to be found where historical records indicated that stocking of hatchery rainbow trout occurred. The inclusion of intraspecific hybridized populations altered genetic structure by creating an artificial shared ancestry among populations from different drainages and led to higher levels of genetic variation in each of the populations. The threats of fragmentation and hybridization will need to be considered in developing conservation and management policies for redband trout in Idaho.

Rainbow trout *Oncorhynchus mykiss* are one of the most widespread and diverse groups of salmonids in North America. In the western United States, Behnke (1992) has identified up to six major subspecies of rainbow trout and Currens et al. (2009) indicated that the greatest evolutionary divergence was between three major river systems (Sacramento, Klamath, and Columbia rivers). The Columbia River redband trout *O. m. gairdnerii* is a major assemblage of rainbow trout found primarily in the Fraser and Columbia rivers east of the Cascade Mountains, whereas coastal rainbow trout *O. m. irideus* are found to the west (Behnke 1992; Currens et al. 2009). Concerns over species status have led to multiple petitions for listing different groups of *O. mykiss* in the interior Columbia River basin under the U.S. Endangered Species Act (ESA; Rhew 2007). In 1997, the anadromous form of Columbia River basin *O. mykiss* (i.e., steelhead) was listed as threatened under the ESA (USFWS 1997). The nonanadromous form was originally included in this listing but was eventually excluded (USFWS 1997). As a result, redband trout above anadromous barriers are managed as independent management units (Western Native Trout Initiative, www.westernnativetrout.org). A tremendous amount of ecological and genetic research has focused on Columbia River

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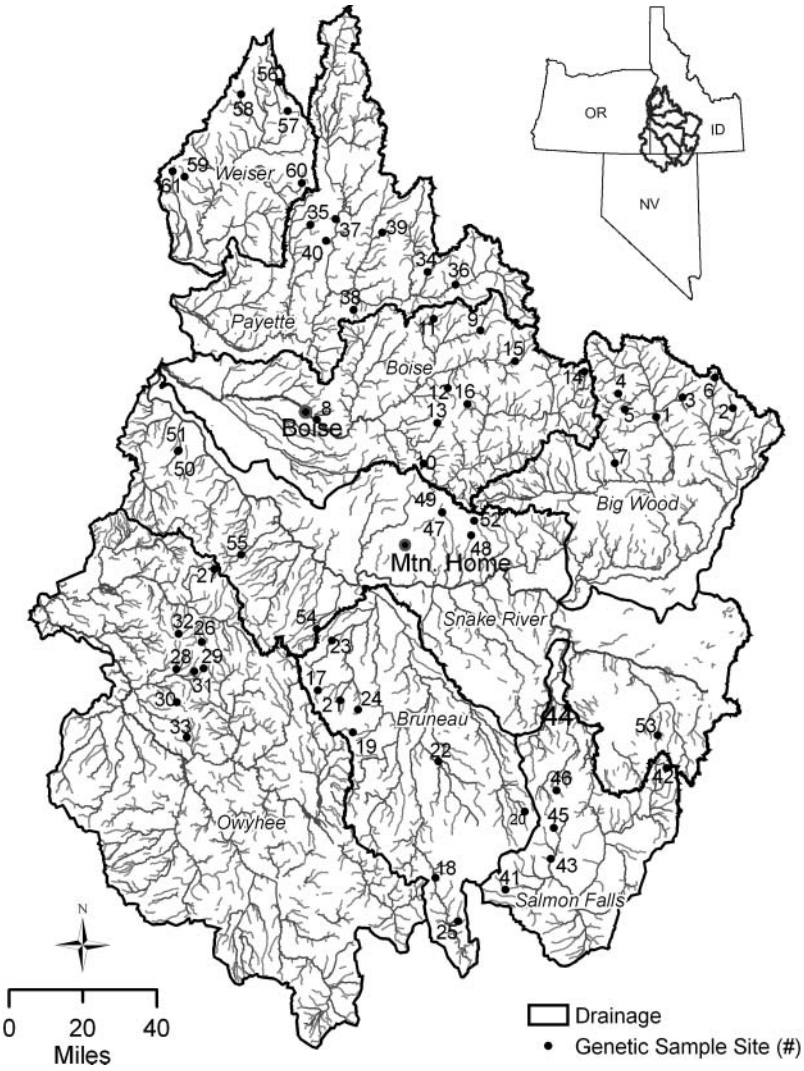

FIGURE 1. Map of the upper Snake River basin showing the sampling locations of redband trout (numbers correspond to those in Table 1) and watershed delineations.

steelhead (Busby et al. 1996; Narum et al. 2006; Currens et al. 2009; Nielsen et al. 2009), but considerably less is known about the nonanadromous counterpart.

Population genetic structure has been investigated in many salmonid species in order to make inferences about population viability (Wenburg et al. 1998; Pritchard et al. 2009). However, genetic structure is often influenced by a range of current and historical factors that can collectively influence patterns of diversity across the landscape. Evolutionary history, selection, and population stability are underlying historical factors (Avise 1994; Waples et al. 2008), whereas contemporary levels of gene flow are often based on the presence of barriers (Neraas and Spruell 2001; Small et al. 2007), the degree of geographic separation (Koizumi et al. 2006), life history behaviors (Neville et al. 2006), and environment. Stocking fish of hatchery origin can further influence population genetic structure by increasing stray rates or creating a shared common ancestry among disparate populations (Hindar et al. 1991; Eldridge and Naish 2007; Hansen et al. 2009). Determining the influences of these factors on observed population structure is essential for sound conservation and management.

In this study, we focus on redband trout above Hells Canyon Dam, a reach hereafter referred to as the upper Snake River basin, Idaho (Figure 1). This area can be broadly divided into two regional macrohabitats: desert and montane. In the desert region, elevations range from 750 to 2,560 m and the landscape is predominately vegetated with sagebrush *Artemesia* spp. in the lower elevations and western juniper *Juniperus occidentalis* and Douglas-fir *Pseudotsuga menziesii* in the higher elevations (Zoellick et al. 2005). Maximum stream temperatures typically fluctuate from 18°C to 26°C during the summer months but have been recorded as high as 30–32°C in some reaches (Zoellick 1999; Meyer et al. 2010). These temperatures exceed the thermal tolerance previously reported for rainbow

TABLE 1. Sample location and identification number (see Figure 1), sample size, genetic diversity, and mean q-values for the 61 sample locations in this study. Abbreviations are as follows: H_e = expected heterozygosity, A = allelic diversity, and A_R = allelic richness. Coastal q-values in bold italics indicate intraspecific hybridization.

No.	Watershed	Sample location	Stocked?	Sample size	Interior q	Coastal q	H_e	A	A_R
1	Big Wood River (montane)	Big Wood River	Yes	28	0.16	***0.84***	0.72	7.62	5.14
2		Copper Creek	Yes	29	0.33	***0.67***	0.59	6.77	4.44
3		East Fork Big Wood River	Yes	21	0.33	***0.67***	0.64	6.38	4.78
4		North Fork Thompson Creek	No	25	0.16	***0.84***	0.66	6.15	4.38
5		Red Warrior Creek	No	59	0.19	***0.81***	0.67	6.08	3.96
6		Little Wood River	Yes	14	0.33	***0.68***	0.52	4.38	3.59
7		Willow Creek	Yes	29	0.31	***0.70***	0.73	7.08	4.85
8	Boise River (montane)	Boise River	Yes	31	0.18	***0.82***	0.75	9.85	5.70
9		Johnson Creek	Yes	58	0.98	0.02	0.69	8.77	5.06
10		South Fork Boise River	Yes	23	0.25	***0.75***	0.78	8.31	5.74
11		Pikes Fork Creek	No	57	0.97	0.03	0.70	8.62	5.08
12		Roaring River	No	57	0.98	0.02	0.72	8.31	5.01
13		Smith Creek	Yes	56	0.31	***0.69***	0.70	7.62	4.66
14		Upper Big Smokey Creek	Yes	30	0.98	0.02	0.70	7.15	4.98
15		Middle Fork Boise River	Yes	45	0.96	0.04	0.73	8.15	5.01
16		Whiskey Jack Creek	No	52	0.95	0.05	0.69	8.08	5.01
17	Bruneau River (desert)	Big Jacks Creek	No	29	0.94	0.06	0.71	6.85	4.71
18		Bruneau River	Yes	18	0.96	0.04	0.74	7.00	5.28
19		Crab Creek	No	35	0.99	0.01	0.48	3.23	2.71
20		Deer Creek[a]	Yes	57	0.99	0.01	0.71	7.31	4.65
21		Duncan Creek[a]	No	73	0.98	0.02	0.63	6.46	4.09
22		Jarbidge River	Yes	46	0.68	***0.32***	0.79	10.77	6.25
23		Little Jacks Creek	No	64	0.95	0.05	0.57	5.85	3.58
24		Wickahoney Creek	No	49	0.93	0.07	0.61	4.77	3.76
25		Willow Creek[a]	No	33	0.99	0.01	0.63	7.23	4.44
26	Owyhee River (desert)	Indian Creek	Yes	30	0.99	0.01	0.71	6.85	4.82
27		Jordan Creek[a]	Yes	55	0.79	***0.21***	0.75	9.31	5.32
28		Juniper Creek	No	30	0.95	0.05	0.72	8.23	5.11
29		North Fork Owyhee River	No	29	0.99	0.01	0.61	4.46	3.66
30		Squaw Creek	No	29	0.97	0.04	0.77	7.85	5.52
31		Unnamed Trib of Owyhee River	No	28	0.99	0.01	0.67	5.46	4.17
32		Williams Creek[a]	No	82	0.96	0.04	0.71	7.23	4.69
33		Petes Creek	No	29	0.82	***0.18***	0.73	7.85	5.26
34	Payette River (montane)	Clear Creek	Yes	22	0.96	0.04	0.67	7.31	5.02
35		Second Fork Squaw Creek	Yes	31	0.78	***0.22***	0.76	9.38	5.96
36		Eight Mile Creek	No	29	0.96	0.05	0.69	8.00	5.09
37		Fawn Creek	No	30	0.98	0.02	0.66	6.23	4.48
38		Longs Creek	No	30	0.96	0.04	0.63	5.92	4.37
39		Silver Creek	Yes	39	0.96	0.04	0.61	7.69	4.41
40		Tripod Creek	Yes	27	0.46	***0.54***	0.74	6.62	5.00
41	Salmon Falls Creek (desert)	Cottonwood Creek	Yes	34	0.98	0.02	0.73	7.54	5.16
42		Middle Fork Shoshone Creek	Yes	23	0.97	0.03	0.67	6.31	4.56
43		North Fork Salmon Falls Creek	No	30	0.95	0.05	0.74	7.77	5.28
44		Salmon Falls Creek	Yes	40	0.23	***0.77***	0.74	8.62	5.38
45		Shack Creek	No	30	0.98	0.02	0.67	5.31	4.14
46		Upper Cedar Creek	Yes	29	0.95	0.05	0.62	5.77	4.22

(Continued on next page)

TABLE 1. Continued.

No.	Watershed	Sample location	Stocked?	Sample size	Interior q	Coastal q	H_e	A	A_R
47	Snake River (desert)	Bennett Creek	No	30	0.99	0.01	0.66	6.31	4.65
48		Cold Spring Creek	Yes	61	0.97	0.04	0.60	5.85	3.96
49		Dive Creek	No	38	0.99	0.02	0.66	6.69	4.73
50		Jump Creek (above falls)	No	43	0.99	0.01	0.51	3.15	2.71
51		Jump Creek (below falls)	No	57	0.69	***0.31***	0.72	5.92	4.44
52		Little Canyon Creek	Yes	32	0.98	0.02	0.70	6.77	4.66
53		McMullen Creek	No	27	0.97	0.03	0.65	5.85	4.26
54		Shoofly Creek[a]	No	30	0.96	0.04	0.61	5.46	3.79
55		Sinker Creek	No	27	0.87	0.13	0.71	6.92	4.76
56	Weiser River (montane)	Beaver Creek	Yes	29	0.75	***0.25***	0.77	9.92	6.11
57		East Fork Weiser River	Yes	28	0.97	0.03	0.73	6.69	4.91
58		Hornet Creek	Yes	29	0.98	0.02	0.71	7.62	4.95
59		Keithly Creek	No	16	0.95	0.05	0.74	7.08	5.40
60		Little Weiser River	Yes	30	0.98	0.02	0.71	8.15	5.32
61		Upper Manns Creek	Yes	31	0.99	0.02	0.69	7.23	4.81

[a]Pooled samples.

trout (26.9–29.8°C: Lee and Rinne 1980; Beitinger et al. 2000). These observations have created interest in whether fish inhabiting these waters are locally adapted to unusually harsh conditions for salmonids (Behnke 1992; Cassinelli and Moffitt 2010) and should be considered as a distinct population segment for conservation and management. Montane streams in this area typically have larger substrate, higher gradient, and more canopy covering from conifer trees and exist at higher elevations (Meyer et al. 2010). Water temperatures are usually several degrees cooler in montane streams than in desert streams during the summer months (Cassinelli and Moffitt 2010; Meyer et al. 2010). In the desert drainages, ephemeral stream flows during drought years and high water temperatures that exceed lethal levels may lead to increased fragmentation relative to more moderate conditions in montane streams.

Anthropogenic activities may also have affected genetic diversity and the genetic structure of redband trout in the upper Snake River basin. In the past 100 years, numerous dams have been built that have blocked the anadromous form of redband trout and isolated populations from one another, starting in 1890 with irrigation dams on the lower Bruneau River and culminating with the completion of the three Hells Canyon dams on the Snake River in the 1960s. The loss of connectivity and anadromy throughout the upper Snake River basin and its effect on genetic diversity has not been extensively addressed. Genetic diversity has only been described for a limited number of upper Snake River populations in Idaho as part of larger studies focusing on steelhead (Narum et al. 2008; Nielsen et al. 2009). Nonnative hatchery trout have also been stocked throughout the upper Snake River basin since the early 1900s. In 2001, the Idaho Department of Fish and Game (IDFG) adopted a policy where only hatchery rainbow trout that are treated to induce sterility are stocked in flowing waters (Kozfkay et al. 2006), but hybridization could have been an outcome from these historical stocking events. Regional assessments are needed to determine how genetic variation is partitioned across the landscape, within hybridized and nonhybridized populations, and to determine how much diversity found within steelhead is still present in the landlocked resident form.

This study addresses genetic diversity of resident *O. mykiss* in the upper Snake River basin. The following objectives and predictions were used as a framework for our analyses: (1) we analyzed population structure at various scales with the prediction that significant spatial structuring would occur at a hierarchical stream network scale as found for other salmonids; (2) we compared genetic structure and diversity among the two regions with the prediction that structure would be greater and diversity would be lower in the desert populations due to thermal barriers and greater degrees of habitat fragmentation; and (3) we compared genetic diversity and structure among hybridized (intraspecific) and nonhybridized populations with the prediction that intraspecific hybridization with nonnative rainbow trout would lead to a breakdown in genetic structure.

METHODS

Sampling and DNA extraction.—During 2001–2005, IDFG personnel collected 3,000 redband trout fin clips representing a mix of age-classes from 150 sample sites in the upper Snake River basin. For this study, 2,233 fin clips were analyzed from sample locations encompassing eight drainages throughout the montane and desert habitats in the upper Snake River, Idaho (Table 1; Figure 1). We divided study sites into desert or montane streams by grouping all streams within the major river drainages north of the Snake River (i.e., the Weiser, Payette, Boise, and Big

Wood rivers) into the montane category, while all the remaining drainages were grouped into the desert category (Meyer et al. 2010). This division corresponds well with differences in geology, vegetation, and precipitation (Orr and Orr 1996) as well as stream habitat (Meyer et al. 2010). Small tributaries of the main-stem Snake River were comprised of high desert habitats (Li et al. 1994) and were lumped into a Snake River drainage grouping. Of the fin clips analyzed, 1,300 samples were located within desert sites and 933 samples were located within montane sites. Temporal samples were taken from one location (Duncan Creek) and some locations were separated by short fluvial distances (<11 km; Williams, Willow, Deer, Jordan, and Shoofly creeks). These collections were analyzed (following the methods described below) to determine whether they could be pooled by tributary. Samples were stored in 100% nondenatured ethanol until DNA extraction. The DNA was extracted using a salt–chloroform method described by Paragamian et al. (1999).

Microsatellite genotyping.—Thirteen polymorphic microsatellite loci were amplified with fluorescently labeled primers: *Oki23* (Genbank accession number AF272822), *Ssa289* (McConnell et al. 1995), *Omy1011* (P. Bentzen, unpublished), *Oke4* (P. Bentzen, Dalhousie University, unpublished), *Ssa408* (Cairney et al. 2000), *Ssa407* (Cairney et al. 2000), *Ots4* (Banks et al. 1999), *One*μ*8* (Scribner et al. 1996), *Ogo1a* (Olsen et al. 1998), *Omy27* (Heath et al. 2001), *Ogo4* (Olsen et al. 1998), *Omy325* (O'Connell et al. 1997), and *One*μ*14* (Scribner et al. 1996). These loci were chosen from a larger group of loci genotyped by Nielsen et al. (2009) and Stephenson et al. (2009). Amplification reaction conditions and polymerase chain reaction (PCR) cycling profiles are available from the authors upon request. The PCR products were separated electrophoretically with an ABI 3100 automated sequencer (Applied Biosystems) platform: PCR products from multiplex 1 (*Oki23*, *Ssa289*, *Omy1011*, *Oke4*, *Ssa408*, *Ssa407*) were electrophoresed together; PCR products from multiplex 2 (*Ots4*, *One*μ*8*, *Ogo1a*, *Omy27*) were electrophoresed together; and PCR products from multiplex 3 (*Ogo4*, *Omy325*, *One*μ*14*) were electrophoresed together. Fragments were sized against GS500 LIZ size standard (Applied Biosystems) with GeneMapper 3.5 software (Applied Biosystems).

Statistical analyses.—In the locations where sampling was conducted in multiple years or over short fluvial distances, an analysis of molecular variance (AMOVA) was performed with ARLEQUIN version 2.1 to evaluate the amount of genetic variation attributable to differences within and between locations (Schneider et al. 2000). Overall, the amount of genetic variation within locations was low (3.3% or lower) so these collections were pooled for subsequent analyses (Table 1). Each population was tested for Hardy–Weinberg equilibrium and linkage disequilibrium with Genepop on the Web (Raymond and Rousset 1995). A Bonferroni correction was used to adjust significance for multiple comparisons for both tests (Rice 1989). An alpha value of 0.05 was chosen for statistical significance for all analyses.

Hybridization.—All populations were initially analyzed to determine the extent of intraspecific hybridization. Stocking records from IDFG were queried to determine which rainbow trout strains were stocked in the sample areas from 1929 to the present. This query revealed that the Mt. Whitney, Mt. Lassen, Hayspur, Eagle Lake, domestic Kamloops, and Troutlodge strains as well as an unspecified strain had been stocked within the sample areas. We were able to obtain 622 fin clips (Table 1) from the following coastal hatchery strains of *O. m. irideus*: Kamloops, Erwin, McConaughy, Arlee, Eagle Lake, Fish Lake, Shasta, Mt. Lassen–Donaldson, Mt. Lassen–Hilderbrand, and Mt. Whitney, as well as the Hayspur strain, which is a mix of coastal and interior fish (Brunelli et al. 2008). All samples were extracted and genotyped with the methods previously outlined.

The Bayesian method of STRUCTURE 2.1 was used to determine whether a sample site had been affected by any past stocking of the reference rainbow trout hatchery strains (Pritchard et al. 2000; Hansen et al. 2001; Small et al. 2007; Stephens 2007). The number of clusters (K) was set to two, assuming the samples would clearly divide into coastal and inland clusters (Blankenship et al., in press) and hybrids would share ancestry in both groups. Ten independent runs of $K = 2$ were run at 100,000 Markov chain Monte Carlo (MCMC) repetitions and 100,000 burn-in steps. The FullSearch algorithm within CLUMPP 1.1.2 (Jakobsson and Rosenberg 2007) was used to combine results across all replicate analyses. The level of admixture was measured as the mean of the individual admixtures from each population. Populations with greater than 10% ancestry in the coastal cluster were classified as hybridized. This threshold was set based upon the simulations of Vaha and Primmer (2006) and Sanz et al. (2009). The individual q-values in STRUCTURE were used to generate a hybrid index for the hybridized populations identified above. A q-value of 0.0 refers to inland redband trout while a q-value of 1.0 refers to coastal rainbow trout. All intermediate q-values indicate hybridized individuals.

All montane sites and two desert sites (lower Jump and Shoofly creeks) were also screened for Yellowstone cutthroat trout *O. c. bouvieri* hybridization since stocking records also revealed that these nonnative fish had been stocked in the area. Six diagnostic nuclear markers were amplified with fluorescently labeled primers: *Om55*, *Occ34*, *Occ36*, *Occ37*, *Occ38*, *Occ42* (Ostberg and Rodriguez 2002, 2004). All six loci were amplified together in one PCR reaction. Amplifications were performed in 10-μL reaction volumes consisting of 5 μL of QIAGEN Multiplex PCR Master Mix (final concentration 1×), 1 μL of primer cocktail (all forward and reverse primers at 100 μM concentration combined together), 3 μL of DNase–RNase-free water, and 1 μL of DNA template (varying concentrations). Fragments were sized against GS500 LIZ size standard (Applied Biosystems) with GeneMapper 3.5 software (Applied Biosystems). Designations between pure parental types, F_1 hybrids, and backcrossed individuals (>F_1 hybrids) were made by evaluating genotypic combinations. Genotypes were

classified as "pure" *O. mykiss* if they were homozygous for rainbow trout at all loci, pure *O. clarkii* if they were homozygous for Yellowstone cutthroat trout at all loci, F_1 hybrids if they were heterozygous for both Yellowstone cutthroat trout and rainbow trout at all loci, and $>F_1$ hybrids if they possessed a mix of heterozygous and homozygous loci.

Genetic diversity and genetic structure.—Genetic diversity was measured by the number of alleles per locus (A), allelic richness (A_R, based on a randomization of a minimum of seven individuals at one locus) and expected heterozygosity (H_e) with FSTAT version 2.9.3 (Goudet 1995). Diversity was calculated at the following scales: population level, all montane populations without hybridized populations, all desert populations without hybridized populations, all hybridized populations, all nonhybridized populations, and all hatchery reference populations. Statistical differences in genetic diversity were measured in two ways. With FSTAT version 2.9.3, a permutation test with 5,000 permutations was used to statistically compare allelic richness and heterozygosity among the following groups: (1) among hybridized and nonhybridized populations, (2) among the eight watersheds, and (3) among regions (montane, desert), both with and without hybridized populations. Intraspecific hybridized populations were included in the comparison among watersheds. Since FSTAT does not report allelic diversity in the permutation test, a t-test was used to compare allelic diversity among these same groupings. To determine how genetic diversity was partitioned among these same hierarchical groupings, AMOVA were also performed using Arlequin 2.0.

A permutation approach that used FSTAT version 2.9.3 was performed to compare the genetic differentiation index (F_{ST}) between watersheds and between regions (montane versus desert). An online version of SMOGD version 2.6 (Crawford 2010) was also used to calculate population pairwise D_{est} (Jost 2008), which is a measure of actual genetic differentiation between subpopulations and can outperform G_{ST} (Jost 2008). G_{ST} is the weighted average of F_{ST} for multiple alleles at a locus. D_{est} is also a measure of absolute population differentiation and differs from G_{ST} in that it is based on allelic identities rather than ratios of heterozygosity (Jost 2008). A regression analysis of D_{est} versus allelic diversity and allelic richness was performed. An unrooted neighbor-joining (NJ) tree using Cavalli-Sforza and Edwards' (1967) chord distance (D_{ce}) was used to display the clustering relationship among populations with the software POPULATIONS 1.2.14 (Langella 2001) and TREEVIEW (Page 1996). One thousand bootstrap replicates were performed to evaluate the strength of the associations in the tree.

RESULTS

Hybridization

Cutthroat trout and interspecific hybrids were identified in six of the sample locations. In total, 34 cutthroat trout and interspecific hybrids were found and removed from the following locations: Copper Creek = one $>F_1$; Little Wood River = four cutthroat trout, 15 F_1, 8 $>F_1$; Willow Creek = two cutthroat trout; Johnson Creek = one F_1; South Fork Boise River = two $>F_1$; Roaring River = one $>F_1$. This constituted 1.5% of all fish sampled and was largely confined to one sample location (Little Wood River).

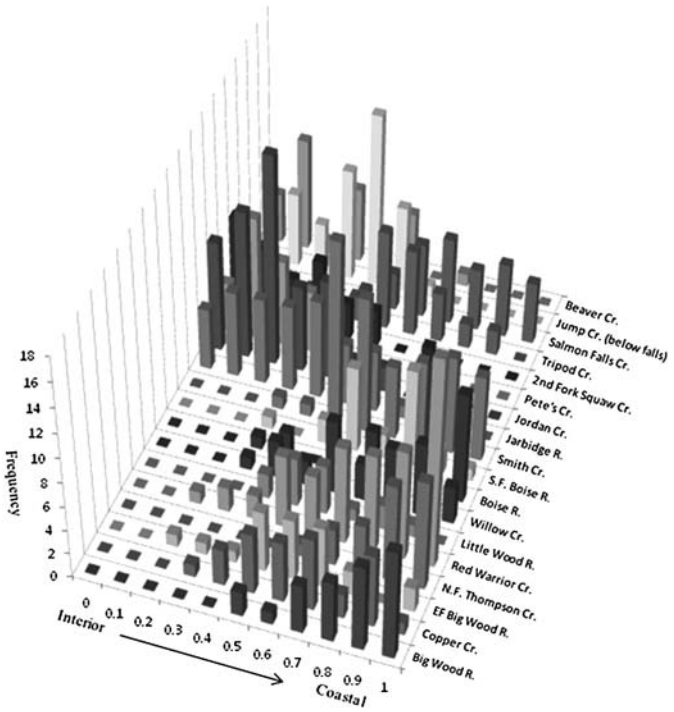

FIGURE 2. Frequency of q-values among individuals in each of the 18 identified hybridized populations; q-values of 0.00 indicate pure native parental types and q-values of 1.0 indicate pure hatchery parental types.

The Bayesian analysis of STRUCTURE was able to delineate populations into coastal and inland *O. mykiss* origin at $K = 2$ and identify intraspecific hybridization. All of the hatchery strains of coastal origin had greater than 95% ancestry in the coastal cluster. Some of the inland populations ($n = 18$) revealed ancestry in the coastal cluster, ranging from 21% to 84% ancestry, and were classified as hybridized (Table 1). Stocking records indicated that 15 of these 18 populations had been stocked with hatchery-origin rainbow trout and that populations at individual sites were 2.6 times as likely to be hybridized if historical records indicated that stocking had occurred. Intraspecific hybridization was more common in the montane sites than desert sites and highest in the Big Wood River watershed, where all of the sampled populations appeared to have been heavily influenced by coastal-origin fish (Figure 2). These populations were skewed towards the coastal end of the index and many coastal parental types ($q = 1.0$) were identified. Intraspecific hybridization in the other watersheds did not appear to be as widespread or high, as some native parental types (q-values = 0.00) were still present and the hybrid index was skewed towards the native end.

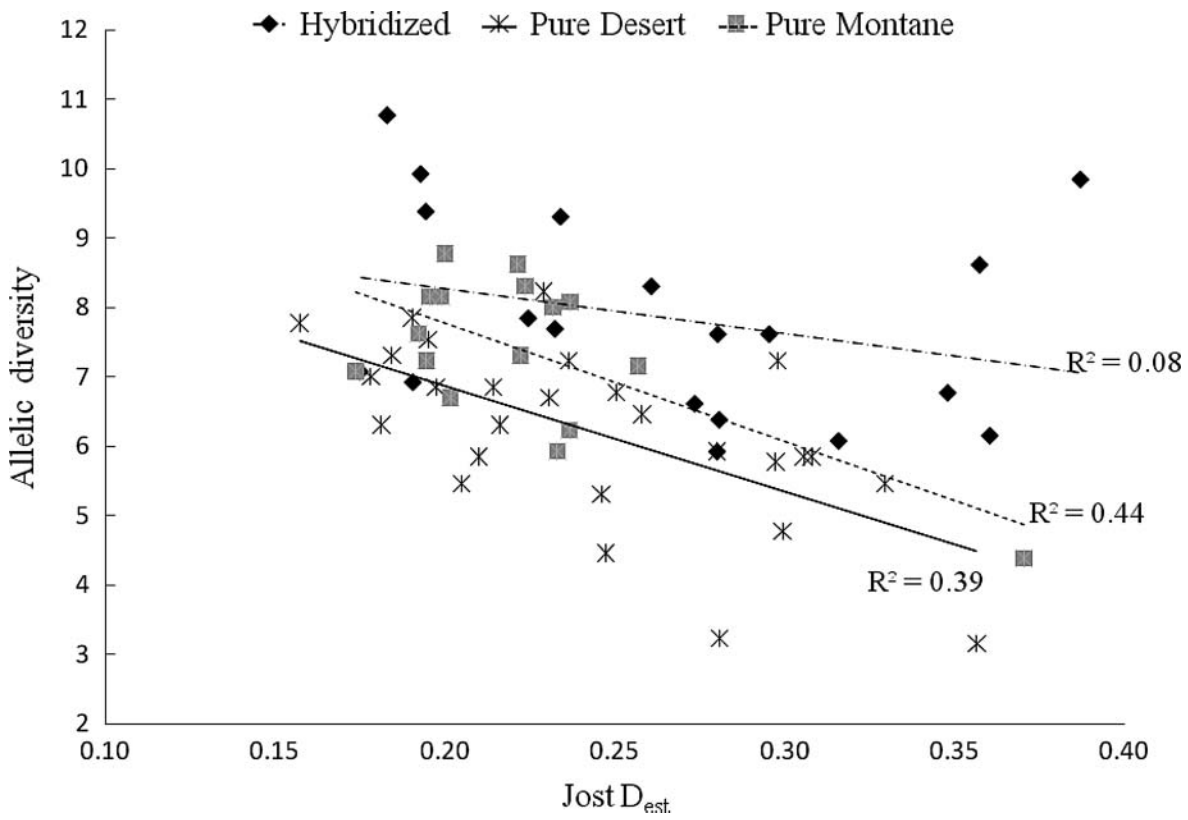

FIGURE 3. Regression analysis of average allelic diversity and Jost D_{est} estimates for each of the 61 populations studied.

Hardy–Weinberg and Linkage Disequilibrium

Prior to a Bonferroni correction, 122 of the 793 tests deviated from Hardy–Weinberg equilibrium. No more than 4 of the 13 loci were rejected per population except for the following instances: Red Warrior Creek (11), Wickahoney Creek (5), Duncan Creek (5), Shack Creek, (6), Tripod Creek (6), and Smith Creek (5), and no more than 28 of the populations were rejected per locus. After a Bonferroni correction, 27 of the 793 tests deviated from Hardy–Weinberg equilibrium. None of the rejected tests were consistently found at a locus but 6 of the 13 loci were rejected in Red Warrior Creek (three deficiencies, three excess). Before a Bonferroni correction, 675 of the 4,780 tests for linkage equilibrium were significant. Only 110 of the tests were significant after a Bonferroni correction and did not cluster around a particular pair of loci. Two populations had many pairs of loci in linkage disequilibrium (Smith Creek, 18 pairs; Red Warrior Creek, 24 pairs), which may be due to ongoing hybridization (see below) or the unintentional sampling of closely related fish.

Genetic Diversity

Genetic diversity varied widely at the population scale. Jump Creek (above a waterfall) had the lowest levels of diversity (3.2 alleles; 51% H_e) and Jarbidge River had the highest levels of diversity (10.8 alleles; 79% H_e). There was no relationship between allelic richness and D_{est} ($R^2 < 0.01$); yet allelic diversity was negatively correlated with D_{est} estimates (desert, $R^2 = 0.39$; montane, $R^2 = 0.44$; Figure 3). At the watershed scale, allelic diversity and allelic richness was lowest in the Snake River ($A = 5.8$, $A_R = 4.2$) and highest in the Boise River ($A = 8.3$) and Weiser River ($A_R = 5.2$). Average H_e ranged from 58% in the Big Wood River drainage to 72% in the Weiser River drainage (Table 2). At the regional scale, the permutation test indicated that montane populations had slightly greater, but not statistically different levels of heterozygosity ($P = 0.22$) and allelic richness ($P = 0.05$) than the desert populations (Table 2). However, a t-test revealed that allelic diversity was significantly higher for montane populations than desert populations when hybridized populations were both included ($t = 2.00$, df $= 59$, $P = 0.02$) and excluded ($t = 2.02$, df $= 40$, $P = 0.002$). Hybridized populations did not have higher levels of allelic richness ($P = 0.12$) or heterozygosity ($P = 0.35$) than nonhybridized populations but did have higher levels of allelic diversity ($t = 2.00$, df $= 59$, $P = 0.02$; Table 2).

Genetic Population Structure

Our results indicate strong spatial structuring of genetic diversity at multiple scales. The AMOVA partitioned a larger amount of genetic variation among regional groupings when

TABLE 2. Hierarchical permutation and AMOVA results for the 61 redband trout populations at various scales. Asterisks denote significance at the 0.05 level.

	Permutation results			AMOVA results				
Group(s)	A_R	A	H_e	Number of groups	Number of populations	Variation among groups	Variation within groups	Variation within populations
All montane populations	4.9	7.5	0.67	2	61	0.62*	12.45*	86.93*
All desert populations	4.5	6.6	0.67					
Nonhybridized montane populations	4.8	7.5	0.69	2	43	1.20*	10.58*	88.22*
Nonhybridized desert populations	4.4	6.2	0.66					
Big Wood River	4.4	6.3	0.58	8	61	2.78*	10.54*	86.67*
Boise River	5.1	8.3	0.71					
Payette River	4.7	7.3	0.65					
Weiser River	5.2	7.8	0.72					
Bruneau River	4.4	6.6	0.66					
Owyhee River	4.9	7.2	0.72					
Salmon Falls Creek	4.8	6.8	0.68					
Snake River	4.2	5.8	0.62					
All hybridized populations	4.9	7.6	0.67	2	61	3.29*	11.41*	85.30*
Nonhybridized populations	4.6	6.7	0.67					

the hybridized populations were removed but still revealed a greater amount of variance explained by population rather than regional or watershed groupings (Table 2). However, all geographic scales were significant at $\alpha = 0.05$. In the NJ tree, hybridized populations clustered together and with the hatchery reference populations (Figure 4). No other patterns of genetic structure were evident from the tree as only some populations clustered with other populations from the same watershed and there was little bootstrap support. Following our prediction that desert populations would experience less gene flow owing to greater degrees of habitat fragmentation, average F_{ST} estimates were significantly higher in the desert populations ($F_{ST} = 0.13$) than among the montane populations ($F_{ST} = 0.07$, $P = 0.03$), although all average population pairwise F_{ST} estimates were moderately large and ranged from 0.08 to 0.23. Levels of genetic differentiation were not significantly different among regions when hybridized populations were included in the comparison ($F_{ST} = 0.12$ in montane, $F_{ST} = 0.13$ in desert; $P = 0.57$).

DISCUSSION

Hybridization

In this study, we used a combination of molecular markers to detect interspecific and intraspecific hybridization. Hybridization with cutthroat trout and hatchery-origin rainbow trout was extensive in a few drainages and was rare and more localized in the other drainages. We found that intraspecific hybrids were more than twice as likely to be found at sites that were previously stocked (45% of the sites) than at sites with no record of stocking (17% of the sites), and although historical stocking records in the study area are probably not 100% accurate, our results demonstrated a positive association between stocking and the presence of hybrids. Nevertheless, the level of coastal introgression varied widely (18% to 84%) and 54% of the stocked streams showed no evidence of intraspecific hybridization. Wishard et al. (1984) detected no effects of hybridization from planting hatchery rainbow trout within eight tributaries of the Owyhee River drainage and suggested that hatchery rainbow trout may not be able to survive as well in those locations. The establishment of introduced fish and breakdown of reproductive barriers that causes hybridization is currently not well understood (Hindar et al. 1991; Williams et al. 1997; Weber and Fausch 2003; Susnik et al. 2004; Small et al. 2007) but is probably multifactorial and related to different stocking histories (e.g., number of years stocked, amount of fish and the strain stocked, size of released fish, fish health, date of last stocking, wild trout densities), naturalization rates of hatchery rainbow trout, and stray rates of hybrids and hatchery rainbow trout (Bennett et al. 2010), as well as environmental variables (Fausch et al. 2001; Bennett et al. 2010). The prevalence of hybrids in certain drainages in this study is most probably due to the higher stocking densities that coincided with easier road access and higher levels of angler use.

Assessing the effect of hatchery stocking on native trout diversity is a high priority but can be especially challenging when it involves intraspecific hybridization (Cordes et al. 2006; Stephens 2007; Simmons et al. 2009). In this study, intra- and interspecific hybrids were treated differently in the analyses. We could reliably detect cutthroat–rainbow trout hybrid individuals as long as hybridization remained low to moderate in the study sites. Using six codominant markers, the probability of

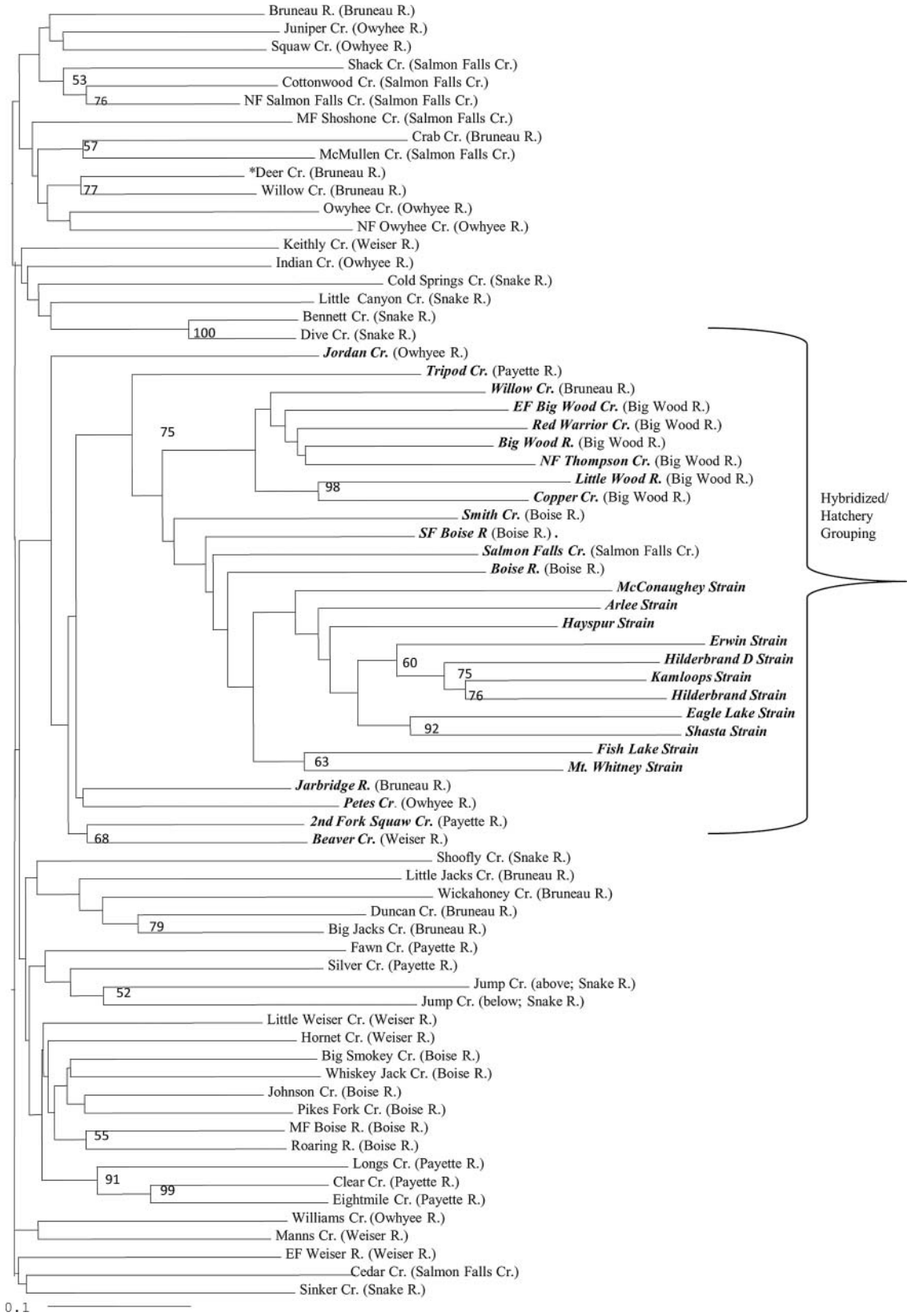

FIGURE 4. Unrooted neighbor-joining dendrogram based on Cavalli-Sforza and Edwards' (1967) chord distance calculated from allele frequencies at 13 microsatellite loci. Populations whose names are in bold italic type clustered with the hatchery reference populations and were classified as hybridized. Bootstrap values greater than 50% are provided.

mistaking a first generation back-crossed individual as a pure-strain fish was less than 1% (Boecklen and Howard 1997). We therefore removed the individual rainbow–cutthroat trout hybrids (but not the study sites) from analyses of genetic diversity and population structure. Even though we were able to determine whether a population was hybridized with hatchery-origin rainbow trout, we were not as confident in the identification of rainbow–redband trout hybrids because of our reliance on allele frequency differences rather than species-specific diagnostic alleles to distinguish hybrids. Vaha and Primmer (2006) indicated that high rates of accuracy could be attained with high divergence and high numbers of loci. While we observed very high divergence (mean $F_{ST} = 0.60$) between the hatchery reference populations and the pure redband trout populations, we believed it would be more conservative to exclude the entire population. The hybrid index also supported the exclusion of entire populations as few native parental types were present in any of the hybridized sample groups. One exception was Sinker Creek, which had an admixture proportion slightly above 10%, but we treated this population as pure in the population comparisons because it did not cluster with other hybridized populations in the NJ tree.

Genetic Structure

In contrast to other *O. mykiss* studies, little regional structuring was observed and the degree of genetic differentiation was much higher for redband trout than has been observed for wild steelhead populations (Clemento et al. 2009; Nielsen et al. 2009). High F_{ST} estimates were observed across all fluvial distances (10–689 km) and the only populations that experienced high levels of gene flow were those separated by short fluvial distances (<11 km), and these were pooled at the onset of the analyses. Population pairwise F_{ST} estimates in this study were more equivalent to those found at the watershed level for steelhead (Heath et al. 2001; Nielsen et al. 2009) or fragmented cutthroat trout populations (Wofford et al. 2005; Cegelski et al. 2006; Neville et al. 2006). Genetic differentiation appears to be largely influenced by genetic drift as evidenced by the long terminal branches in the NJ tree and because the majority of populations did not cluster with other populations from the same watershed. This indicates that life history or other factors are limiting gene flow among populations and that genetic drift has erased most signatures of regional structure. Strong homing or resident life histories can constrain gene flow (Neville et al. 2006). Habitat fragmentation from agricultural and grazing land uses, low water flows, or physical and hydrologic temperature barriers (Zoellick 1999; Zoellick et al. 2005) have also probably led to reduced gene flow among desert populations.

Overall, the desert populations were more differentiated than the montane populations. As a group, the desert populations also had statistically lower levels of allelic diversity than the montane populations, but not allelic richness. As allelic richness was based upon a small sample size, it is hard to infer whether allelic diversity is truly lower in these populations. These regional differences could be due to smaller population sizes in the desert populations, intermittent stream flows prohibiting gene flow between some of the desert streams, or both. For example, in the Bruneau River drainage, Little Jacks, Big Jacks, and Wickahoney creeks experienced significant isolation from other populations in the drainage owing to intermittent flows in the lower portions of the stream. Furthermore, Little Jacks and Big Jacks creeks are probably never connected to the main-stem Snake River owing to habitat alterations and recent stream desiccation. As land use practices continue to alter patterns of perennial stream flow and water temperature, it is likely that the isolation of redband trout populations in southwest Idaho may be exacerbated. Zoellick et al. (2005) indicated that redband trout populations in desert streams at lower elevation were less productive and had slower recruitment following drought years, while populations in higher-elevation desert streams were less affected by these factors. Thus, temporal changes in stream temperature and flow may restrict redband trout to isolated headwater populations and lead to increased fragmentation in some years (Zoellick 1999; Zoellick et al. 2005). Neville et al. (2009) indicated that rainbow trout rapidly recolonized burned areas and found no evidence for reduced genetic diversity in recolonized populations. If the intrinsic potential for dispersal remains strong in these desert populations of redband trout, we would expect to see increased dispersal among sites in high water years. To understand the impact of this dynamic environment on population genetic characteristics, it may be of interest to analyze temporal samples.

The loss of anadromy after dam construction appeared to affect diversity and population structure throughout the upper Snake River basin. In both regions, an inverse relationship between levels of genetic differentiation and allelic diversity was observed, where the most isolated populations retained the least amount of genetic diversity. The AMOVA also indicated that a greater amount of diversity was partitioned by watershed than by region, suggesting some historical structuring of diversity at the watershed level. It is not surprising that genetic drift would lead to reduced diversity in small, resident populations, but these populations were probably not as small and fragmented when anadromous steelhead were present (unless there was historical segregation). We compared genetic diversity in this study with 27 Snake River steelhead populations below Hells Canyon Dam (M. R. Campbell, unpublished data). This comparison revealed that pure redband trout populations had lower levels of average allelic diversity ($A_{average} = 6.72$) than did steelhead populations ($A_{average} = 9.28$), but collectively 93% of the alleles present in the steelhead populations were shared among the current study populations. Our results indicate that all of the redband trout populations retained a subset of the anadromous diversity, which has been partitioned differently throughout the range by genetic drift. This finding is not novel as other studies have also reported that *O. mykiss* populations above artificial barriers contained similar components of the gene pool found in nearby anadromous populations (Nielsen et al. 1997; Deiner et al. 2007;

Clemento et al. 2009). In this study, populations with the highest levels of genetic diversity were the least differentiated, and this could be because they contained more relict alleles. If this was indeed the case, observed levels of genetic differentiation could reflect historical patterns of connectivity more so than current levels of gene flow.

Hybridization created an artificial common ancestry among populations regardless of the amount of hybridization. In the NJ tree, all of the hybridized populations clustered with one another rather than with populations from the same drainage. Also, when hybridized populations were included in the AMOVA and permutation tests, regional differences were not as apparent. When hybridized populations were removed from comparisons, desert populations were more differentiated than montane populations. These results indicate that hybridization decreased genetic differentiation and homogenized genetic diversity through the sharing of nonnative alleles. Loss of genetic variation has been documented to co-occur with a breakdown in genetic structure as populations are replaced with less diverse hatchery stocks (Jug et al. 2005). Hybridized populations in this study also had greater allelic diversity than nonhybridized populations, resulting from the additive effect of mixing native alleles with nonnative alleles. These results indicate that intraspecific hybridization, regardless of the level, can significantly alter diversity levels and population relationships, and many populations could be misinterpreted as having high gene flow or high diversity if a hybrid screen was not run before the analysis. Given this, an understanding of hybridization is necessary to develop sound management practices, not only in determining the conservation value of populations but in making inferences about diversity and gene flow.

The elevated temperatures associated with these high desert drainages have led some researchers to question whether adaptation is also a driving force. Redband trout have been observed in water temperatures that exceed the reported thermal tolerance of rainbow trout and other salmonids (e.g., 27°C, Schrank et al. 2003; 28.5°C, Dunham et al. 2003) and this has prompted several authors to design field and laboratory physiology studies to evaluate the temperature tolerance of redband trout (Gamperl et al. 2002; Rodnick et al. 2004; Cassinelli and Moffitt 2010). Rodnick et al. (2004) found that redband trout displayed a similar upper temperature tolerance (T_{crit}) as other rainbow trout and salmonids but appeared to have the second highest values of metabolic power and maximum metabolic rate at 24°C of published values for salmonids, which may give them a physiological advantage in warmer waters. Cassinelli and Moffitt (2010) evaluated growth, physiology, and lethal temperature maximums for montane and desert redband trout reared together under representative diurnal water temperature cycles and concluded that both were equally tolerant of high temperatures and that redband trout were a highly plastic species. This plasticity may explain why rainbow trout are one of the most widely propagated and distributed species in the world (Fausch et al. 2001). The quality of plasticity or ability to be molded to survive in different environmental conditions is probably a prerequisite for local adaptation to occur (Jensen et al. 2008). The microsatellite markers in this study are presumably neutral and do not have the ability to ascertain selection or adaptive diversity (Nielsen et al. 2009), so other markers may provide more insight. Recently, Narum et al. (2010) used a limited gene scan approach to detect six candidate markers that may be undergoing selection in these differing environments (montane versus desert) and found that populations in the same climatic environment had similar allele frequencies, reflecting a pattern not seen with neutral loci. Further validation of these markers within controlled environments (Narum et al. 2010) and quantitative classifications of many habitat features within these streams will be necessary for linking possible adaptation to gene function.

Conservation and Management Implications

The majority of genetic diversity was partitioned at the population scale. Therefore, management of redband trout in southwest Idaho should maintain a number of populations within each watershed to ensure adequate representation of genetic variation. This diversity probably was historically partitioned at the watershed scale when steelhead were present, except in isolated, headwater streams where only resident redband trout may have resided. Given the current picture of diversity, the reconnection of important migratory corridors and habitat improvement projects may increase dispersal among populations. For example, current land uses and irrigation diversions prevent some trout populations from being connected via the Snake River (Zoellick 1999), so more localized habitat restoration projects or carefully considered translocations may benefit desert redband trout. In the desert streams, connectivity not only refers to habitat reconnection but also to removing thermal barriers (Zoellick and Cade 2006).

While increasing connectivity can have many positive outcomes (increased gene flow, increased abundance, life history diversity), the potential spread of hybrids can be a negative outcome (Peterson et al. 2008; Fausch et al. 2009). Reconnecting tributaries to the main-stem Snake River could increase the likelihood of intraspecific hybrids spreading into pure-strain populations, so hybridization would have to be taken into account before any reconnection project. The detection of interspecific hybridization between stocked cutthroat trout and native redband trout is also of concern. While cutthroat trout hybridization was low, the detection of F_1 hybrids in two of the sample locations suggests that interspecific hybridization is ongoing. Owing in part to these data, stocking cutthroat trout in many headwater lakes has been discontinued until sterile fish can be produced (J. Dillon, IDFG, personal communication). In contrast, intraspecific hybridization more probably occurred in past decades when nonsterile hatchery strains of coastal rainbow trout were stocked. Hardy–Weinberg expectations were met in all of the populations, except for Red Warrior Creek, indicating that equilibrium has been regained and recent hybridization events have not occurred (Cordes et al. 2006). While

hybridization may not be ongoing in the majority of the locations, the replacement of native diversity with hatchery-origin rainbow trout is of concern. We recommend continued monitoring hybridization in these populations and others nearby to determine whether the levels are stable, increasing, or declining.

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